

MATING BEHAVIOR OF THE SOCIALLY-PARASITIC
ANT *POLYERGUS BREVICEPS*:
THE ROLE OF THE MANDIBULAR GLANDS

BY HOWARD TOPOFF¹ AND LES GREENBERG²

INTRODUCTION

The ant genus *Polyergus* consists of four species, all of which are obligatory social parasites of the related formicine genus *Formica*. There are two principal behavioral contexts in which host workers form a social attachment to the slave makers. The first is inside the *Polyergus* nest, where the parasitic workers, queen, and brood must be fed and otherwise cared for by *Formica* individuals. These slave ants are obtained during group raids, in which a swarm of *Polyergus* workers invades a nest of *Formica*, disperses the adult workers and queen, and carries off the pupal brood (Topoff et al., 1984, 1985). A portion of this raided brood is reared in the slave-maker nest, and workers which eclose perform their typical functions (i.e., foraging, feeding, nest defense) as permanent members of the parasite colony.

The second context of social-bond formation occurs during colony founding by *Polyergus* queens. Because queens are not capable of rearing even their first brood, a newly-mated female penetrates a colony of *Formica*, kills the resident queen, and becomes accepted by the *Formica* workers (Topoff et al., in press).

The mating and post-mating behavior of *Polyergus* queens includes several adaptations for locating colonies of *Formica*. After the mating flight of *P. lucidus*, for example, dealate queens often return to a *Polyergus* colony and follow subsequent slave-raid swarms to target colonies (Kwait & Topoff, 1984; Marlin, 1971; Talbot, 1968).

In this manuscript, we report the results of a study on mating behavior of the western slave-making ant *P. breviceps*. Queens of

¹Department of Psychology, Hunter College of CUNY, New York, N.Y. 10021, and Department of Entomology, The American Museum of Natural History, New York, N.Y. 10024.

²Department of Entomology, Texas A & M University, College Station, TX 77843
Manuscript received by the editor December 29, 1987.

this species have an even more efficient mechanism for reaching *Formica* colonies. Instead of a mating flight, winged *P. breviceps* queens copulate during the slave raids. The queens stop running momentarily, attract males with a pheromone produced in the mandibular glands, mate and shed their wings, and then promptly continue with the raiding workers to the target nest.

PRELIMINARY FIELD STUDIES

This study was conducted at the Southwestern Research Station, located 5 km west of Portal, Arizona. At an altitude of 1646 m, the ground in this habitat is covered with bunch grass and contains extensive leaf litter from alligator juniper, Arizona oak, and Chihuahuahua pine. A total of six colonies were monitored during the period when sexual individuals were present (July 15–August 10, 1987). During each day, three of the colonies were observed continuously from 1400 to 1800 hr (MST).

Males and queens of *P. breviceps* appeared outside the nest 1–2 hr before raiding, along with the milling workers, and ran around the nest perimeter. The males flew off intermittently during milling, until the onset of raiding. But we only observed two instances of flight by *Polyergus* queens, as compared with over 100 queens (from the six colonies) that mated in slave-raid swarms with no prior flight. This contrasts markedly with the related species *P. lucidus*, in which queens fly off 5–30 min after the males' departure (Marlin, 1971; Talbot, 1968). The number of alate queens running in the raid swarms ranged from 8–29 ($n = 9$ observations, $\bar{x} = 18$). Although we could detect no obvious behavioral display, some queens vibrated their wings while running. At varying distances from the home nest, the queens ceased running. This inactivity was immediately followed by the appearance of highly-aroused males. The males ran erratically in circles, either around the queen or slightly off to the side. They often mounted each other. When a male finally copulated with the queen, she immediately pulled off her wings and continued running in the slave-raid swarm towards the target *Formica* nest.

Preliminary field tests showed that squashing a *Polyergus* queen near a raiding column attracted males. To determine the source of the chemical attractant, queens were collected from slave-raid swarms and frozen. Using microdissection scissors, we sectioned

each queen into head, thorax and abdomen. These three body parts were put into separate vials and taken to *Polyergus* nests. We then used the blunt end of a small plastic paintbrush to squash the body part on a small rock near a raiding swarm. For three tests (each with a different colony), males responded to the squashed heads by running in circles and mounting each other. No males appeared when we crushed the thorax or abdomen.

LABORATORY STUDIES

Methods

To determine the source of the queen's sex pheromone, glands dissected from the heads of *P. breviceps* queens were presented to males confined in a laboratory enclosure. Because of its large size, the primary candidate for the sex attractant was the mandibular gland and its reservoir. Dissections were done in a wax-lined petri dish, filled with mineral oil (to retard evaporation of the volatile pheromone). First, the cuticle around the queen's mandible was cut (or removed). By holding the queen's head firmly with forceps and then gently pulling on the mandible, we were able to remove the mandibular gland. Only those preparations containing the intact glandular reservoir were used for bioassays. As a control, we used the remains of the head after the mandibular glands were removed.

The behavioral tests were conducted with seven *Polyergus* males, confined together in a plastic box (16.5 cm × 12.5 cm × 6.5 cm high). The lid of the box had a large opening (10 cm × 8 cm), covered by a mesh screen. Additional ventilation was provided by holes (2.5 cm diam) cut into opposite ends of the box. Rubber tubing (5.0 cm long and capped with nylon mesh) was placed tightly into these holes. All tests were conducted under artificial light, both to maintain the experimental chamber at 29°C, and to inhibit the males' typical approach response to the day-time sky. At the start of a test, one mandible with the attached gland and reservoir (or remaining head) were held in forceps near the rubber tube's outer opening. A constant current of air through the box was provided by a fan placed 2 m from the chamber. After recording the number of active males during a 10-s interval, each preparation was crushed with the forceps. We then recorded the number of males active during a second 10-s interval, after which the preparation was removed. In all cases, we recorded a response as positive if the males

exhibited at least one of the following behaviors: flying; running in circles; or mounting other males. Both glands from each queen were tested (except when the reservoir or mandible broke during dissection), followed by a similar test with the remaining head. The interval between all tests was 5 min. Glands and heads from 10 different queens (5 alate, 5 dealate) were used. Finally, we also tested two dissected maxillary and postpharyngeal glands, as well as squashed heads from two workers and two males. Unfortunately, by the time this laboratory study was established, it was the end of the reproductive season. As a result, the same 7 *Polyergus* males had to be used for all tests.

Results

Table 1 shows the results for the 10 queens and 7 males. The proportion of males active before squashing the mandibular gland reservoir was 0.02 (summed over all trials; $n = 126$). Afterwards the proportion was 0.84. For tests with squashed queen heads after removal of the mandibular glands, the proportion of males active was 0.27 ($n = 56$ cases). The latter activity may be due to occasional leakage of mandibular substance into the head during the dissection. The behavior of the males during positive tests was very similar to their responses to crushed queen heads in the field. Although sustained flight was impossible in the small plastic enclosures, the squashed mandibular glands immediately caused the males to run excitedly in circles inside the box, and they occasionally even mounted each other. The mandibular glands of dealate queens (which were collected near raiding columns or from *Polyergus* nests) were as effective in exciting males as those of alate queens. Finally, no activity by the experimental males was elicited either by dissected maxillary and postpharyngeal glands, or by squashed heads from two workers and two males.

DISCUSSION

The mating behavior of *P. breviceps* queens is ecologically similar to the "female calling syndrome" that is commonly found in primitive and socially-parasitic ants (Hölldobler & Bartz, 1985). A principal characteristic of this process is that new queens do not disperse widely; instead, they remain close to their nest of origin and secrete male-attractant pheromones. Also typical of this behavior is the production each year of relatively few reproductives, and nuptial

Table 1. Response of *Polyergus* males to sex pheromone of queens. Values shown are the number of males ($n = 7$) active before and after squashing either the mandibular gland or the remaining head.

Queen #	Gland #	No. males responding to mandibular glands		No. males responding to head without mandibular glands	
		Before	After	Before	After
1 (D)	1	0	7	---	---
	2	---	---	---	---
2 (A)	1	1	6	0	4
	2	0	5		
3 (D)	1	0	6	0	1
	2	0	6		
4 (D)	1	0	7	0	1
	2	0	7		
5 (D)	1	0	6	1	1
	2	1	4		
6 (D)	1	0	5	0	1
	2	0	6		
7 (A)	1	0	7	0	1
	2	0	6		
8 (A)	1	0	2	0	5
	2	0	7		
9 (A)	1	0	7	---	---
	2	---	---		
10 (A)	1	0	5	0	1
	2	1	7		
Totals		3	106	1	15

(A): alate; (D): dealate

---: test not conducted

flights that are not well synchronized between the sexes (Buschinger, 1975).

To date, the most detailed studies of sexual calling behavior in parasitic ants have been conducted on the myrmicine tribe Leptothoracini. Thus in both *Harpagoxenus canadensis* and *H. sublaevis*, queens attract males by poison-gland secretions released from the extruded stinger (Buschinger & Alloway, 1979). In species of ants in which mandibular glands are the source of sex attractants, it is typically males that release the pheromone. In myrmicine harvester ants of the genus *Pogonomyrmex*, for example, males are attracted

in large numbers by mandibular-gland secretions that other males discharge upon arrival at the mating arena. Queens are subsequently "lured" to these arenas by the collectively-secreted male pheromone (Hölldobler, 1976, 1984). In the formicine ant *Camponotus herculeanus*, mass flights of winged queens from the nest are also stimulated by secretions from the males' mandibular glands (Hölldobler & Maschwitz, 1965). Although queen honey bees secrete a sex pheromone (trans-9-keto-2-decenoic acid) from their mandibular glands (Butler, 1971), *Polyergus breviceps* is the first species of ant in which the queen's sex-attractant pheromone has been traced to the mandibular glands.

In an extensive review of flight activities, Kanno (1963) reported a distinct tendency for ground mating in formicine ants. In *Formica rufa* and *F. subnuda*, for example, ground mating occurs after a nuptial flight. But in *Formica subintegra* and *F. montana*, winged females typically remain near their nest, with approaching males forming a "ground swarm" that terminates in mating. Alate queens of *Polyergus breviceps* have apparently adapted this behavioral pattern to a parasitic mode of life, especially their inability for independent colony foundation (Topoff et al., in press). By running and mating in slave-raid swarms, mated queens of *P. breviceps* arrive at colonies of *Formica* whose workers and queen are scattered across the substrate. Such disorganization in the target colony could facilitate the usurpation of *Formica* colonies by *Polyergus* queens.

REFERENCES

BUSCHINGER, A.

1975. Sexual pheromones in ants. In: Pheromones and defensive secretions in social insects (Ch. Noirot, P. Howse, and G. Le Masne, eds.). Proc. Symp. IUSSI. Pp. 225-233.

BUSCHINGER, A. AND ALLOWAY, T. M.

1979. Sexual behavior in the slave-making ant, *Harpagoxenus canadensis* M. R. Smith, and sexual pheromone experiments with *H. canadensis*, *H. americanus* (Emery), and *H. sublaevis* (Nylander) (Hymenoptera; Formicidae). Z. Tierpsychol. **49**: 113-119.

BUTLER, C. G.

1971. The mating behavior of the honeybee (*Apis mellifera* L.). J. Entomol. **46**: 1-11.

HÖLLDOBLER, B.

1976. The behavioral ecology of mating in harvester ants (Hymenoptera: Formicidae: *Polyergus*). Behav. Ecol. Sociobiol. **1**: 405-423.

HÖLLDOBLER, B.

1984. Evolution of insect communication. In: Insect communication (T. Lewis, ed.). Academic Press, N.Y. Pp. 349-377.

HÖLLDOBLER, B. AND BARTZ, S. H.

1985. Sociobiology of reproduction in ants. In: Experimental behavioral ecology and sociobiology (B. Hölldobler and M. Lindauer, eds.). Sinauer Assoc., Pp. 237-257.

HÖLLDOBLER, B. AND MASCHWITZ, U.

1965. Der Hochzeitsschwarm der Rossameise *Camponotus herculeanus* L. (Hym. Formicidae). Z. vergl. Physiol. **50**: 551-568.

KANNOWSKI, P.

1963. The flight activities of formicine ants. Symp. Genetica et Biologica Italica. **12**: 74-102.

KWAIT, E. AND TOPOFF, H.

1984. Raid organization and behavioral development in the slave-making ant *Polyergus lucidus* Mayr. Insectes Soc. **31**: 361-374.

MARLIN, J. C.

1971. The mating, nesting and ant enemies of *Polyergus lucidus* Mayr (Hymenoptera: Formicidae). Amer. Mid. Nat. **86**: 181-189.

TALBOT, M.

1968. Flights of the ant *Polyergus lucidus* Mayr. Psyche. **75**: 46-52.

TOPOFF, H., COVER, S., GREENBERG, L., GOODLOE, L. AND SHERMAN, P.

(in press). Colony founding by queens of the obligatory slave-making ant *Polyergus breviceps*: the role of the Dufour's gland. Ethol.

TOPOFF, H., INEZ-PAGANI, M., MACK, L. AND GOLDSTEIN, M.

1985. Behavioral ecology of the slave-making ant *Polyergus breviceps* in a desert habitat. Southwest. Nat. **30**: 289-295.

TOPOFF, H., LAMON, B., GOODLOE, L. AND GOLDSTEIN, M.

1984. Social and orientation behavior of *Polyergus breviceps* during slave-making raids. Behav. Ecol. Sociobiol. **15**: 273-279.